Remarks/Arguments

Claims 20 and 23 were amended to insert "test" next to "compound" as suggested by the examiner; and withdrawn claims 8-11 were canceled without prejudice to future prosecution. The amendments to claims 20 and 23 correct an informality noted by the examiner and do not alter the claim scope.

Claims 12, 14, 17, 18, and 22-23 stand rejected as allegedly obvious based on Tomei et al. (Journal of Virology 67(7):4017-4026, July 1993). The rejection is based on Tomei et al. indicating that the HCV NS5 region is cleaved into products of 47 kDa (NS5A) and 65 kDa (NS5B); and Tomei et al. suggesting, based on a GDD sequence, that NS5B "may" act as a viral replicase. The rejection is respectfully traversed.

The prior art speculations concerning a possible role for NS5B and the prior art uncertainty as to whether NS5B is an authentic HCV protein, taken together, do not provide: (1) a motivation to modify Tomei et al. to obtain the claimed assay; or (2) a reasonable expectation of success in modifying Tomei et al. to obtain the claimed assay. Tomei et al. concerns HCV polyprotein processing. Tomei et al. does not describe using NS5B in an assay to look for a RNA-dependent RNA polymerase activity inhibitory compound. The rejection argues for a modification of Tomei et al.

At the time the invention was made, prior art publications expressed uncertainty concerning whether NS5B was a recombinant expression artifact. Such publications need to be considered for what they would have taught the skilled artisan in evaluating predictability and the likelihood of success at the time the invention was made. As noted by the Manual of Patent Examining Procedure (MPEP):

> The test for obviousness is what the combined teachings of the references would have suggested to one of ordinary skill in the art, and all teachings in the prior art must be considered to the extent that they are in analogous arts. Where the teachings of two or more prior art references conflict, the examiner must weigh the power of each reference to suggest solutions to one of ordinary skill in the art, considering the degree to which one reference might accurately discredit another. (Citations omitted.)

(MPEP Rev. 2, May 2004 at 2100-130, last second column to the top of 2100-131.) Applicants are not arguing whether or not NS5B is in fact an expression artifact.

In hindsight, it is known that NS5B provides for RNA-dependent RNA polymerase activity. That the inventors were ultimately successful is irrelevant to whether the skilled artisan would have reasonably expected success at the time the invention was made. *Life Technologies Inc. v. Clontech Laboratories Inc.*, 56 USPQ2d 1186, 1192 (Fed. Cir. 2000).

Additional considerations also demonstrate patentability of the pending claims. Such considerations include prior art noted difficulties in obtaining active HCV RNA-dependent RNA polymerase activity; prior art noted difficulties in successfully purifying HCV RNA-dependent RNA polymerase activity; and a long-felt need for a HCV RNA-dependent RNA polymerase assay to look for polymerase inhibitory compounds.

Successfully purifying HCV RNA-dependent RNA polymerase activity involves obtaining a purified product that retains activity. Claim 12 indicates purification to apparent homogeneity. Claims 14, 17, and 18 depend from claim 12.

Uncertainty Concerning Authentic HCV Proteins

Prior art uncertainty concerning the relevance of recombinantly produced NS5B to authentic HCV proteins is reflected in differences between published results obtained from HCV infected liver cell versus recombinantly processed HCV polyprotein. Uncertainties are also noted in cautionary language used in publications concerning recombinant NS5B.

Tsutsumi et al. (Hepatology 19(2), 265-272, 1994) indicates that HCV in infected liver cells did not produce a protein with approximately the same molecular weight as NS5B, in contrast to a study using a recombinant system that produced NS5B:

> Grakoui et al. (15) reported that two proteins were derived from the HCV-NS5 region: NS5A (58kD) and **C-terminal NS5B (66 to 68 kD)**, when a cDNA encompassing the long open reading frame was used in the vaccinia virus transient-expression assay. The NS5B protein was predicted to contain the RNA-dependent RNA polymerase activity on the basis of the presence of the characteristic Gly-Asp-Asp and surrounding conserved motifs. Although bacterially expressed HCV-NS5 peptide fragment was used for a part of NS5B protein in this study, the molecular size of **HCV-NS5-related antigen detected in human liver was 86 kD** and thus slightly larger than that of NS5B. This discrepancy may have resulted in different host cells, which were cultured mammalian cells in Grakoui's study (15) and were human liver cells in this study. **Furthermore, we observed the products derived from native HCV, whereas Grakoui et al., (15) observed the polypeptide expressed from HCV cDNA in vaccinia virus.** [Emphasis added.]

(Tsutsumi et al. starting at page 269, first column, second paragraph to page 270, first column.)

The magnitude of the different molecular weights for infected human liver versus recombinantly processed HCV NS5 regions point to different proteins and not minor variations. The 86 kD molecular weight noted by Tsutsumi et al. is 21 kD more than the Tomei et al. NS5B weight of 65 kD and 18 kD

more than the upper 68 kD attributed to Grakoui et al. Assuming the average molecule weight of an amino acid is 137, 18 kD provides for over 100 amino acids.[1]

Both Tomei et al. and Grakoui et al. (Journal of Virology 67(3), 1385-1395, 1993) point out that recombinantly produced NS5B may not correspond to a naturally produced product. According to Tomei et al.:

> It is clear, however, that the results obtained with this transient expression system may **not faithfully reproduce the proteolytic events which take place during HCV infection**. It is possible that the level of protein expression obtained in this system may be much higher than normal, affecting important equilibria between precursors and proteases, which in turn may regulate HCV replication and protein synthesis. [Emphasis added.]

(Tomei et al., at page 4025, first column, middle of the third paragraph.) Similarly, Grakoui et al. notes in its concluding paragraph:

> The experiments reported here have given us a preliminary picture of HCV polyprotein organization and processing. However, **this view is far from complete, and additional studies are needed to define polyprotein cleavage sites** and the responsible proteinases and to verify that the products observed in these expression studies are similar to those produced in authentic HCV infections. Such information should prove valuable for expression and characterization of HCV-encoded enzymes as potential targets for antiviral therapy and will allow future studies . . . [Emphasis added.]

(Grakoui et al. at page 1393, first column, third paragraph.)

Apparent Failure, Difficulty Encountered by Others and Long-Felt Need

Additional considerations illustrating the inventive nature of the pending claims include:

- Apparent failure and difficulty encountered by others in successfully purifying HCV RNA-dependent RNA polymerase;
- Apparent failure and difficulty encountered by others in demonstrating the HCV region responsible for RNA-dependent RNA polymerase; and
- A long-felt need for an HCV RNA-dependent RNA polymerase assay to look for polymerase inhibitors. The pending claims are directed to a method of identifying a HCV RNA-dependent RNA polymerase inhibitor.

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[1] Molecular weights for naturally occurring amino acids vary from 75.07 to 204.3 (CRC Handbook of Chemistry and Physics 81st Edition, David Lide Editor, 7.1 CRC Press, 2000-2001.) An average molecular weight of 137 is based on the individual weights of the amino acids and does not take into account the frequency of a particular amino acid in a protein.

Apparent failure and difficulty encountered by others in successfully purifying HCV RNA-dependent RNA polymerase and demonstrating the HCV region responsible for RNA-dependent RNA polymerase is evident based Chung et al. (Hepatology, 16(4), 1992) and WO 97/12033. Chung et al. is an abstract mentioning attempts to obtain HCV RNA-dependent RNA polymerase from liver tissue. Chung et al. references activity obtained with partially purified extracts. Reference to only partially purified activity is consistent with failed attempts to obtain a purified product. Failed purification attempts are evident based on use of different chromatographic techniques and the desirability to obtain a purified enzyme for further study.

Chung et al. also directs the skilled artisan away from using recombinantly produced HCV protein by indicating the use of liver extracts. However, Chung et al. does not provide sufficient information to enable the skilled artisan to reproduce the assay or to verify the assay was successful. Important missing information includes how liver tissue samples were obtained and treated, what conditions were employed for the assay, and which conditions were employed for purification.

WO 97/12033 has a priority date of September 27, 1995, which is about four months after the priority date for the present application. According to WO 97/12033, in the Background of the Invention:

> The non-structural protein designated 5B (NS5B) has been shown to have an amino-terminal sequence SMSY (Ser-Met-Ser-Tyr). The NS5B region encodes a 68kd protein (p68) which contains an internal GDD (Gly-Asp-Asp) motif found in RNA-dependent RNA polymerases of other RNA viruses (Koonin, E. V. (1991) *J. Gen. Virol.* 72:2197-2206). **However, no polymerase activity has been detected for HCV p68.** In fact, the question has been raised that the 5B protein (p68) alone does not encode an active RNA-dependent RNA polymerase enzyme and that another subunit, possibly the NS5A gene product, is essential to catalytic activity. **Prior attempts by the inventors and others to express the NS5B coding region as a fusion protein, using existing expression systems that facilitate purification of the fusion product and specific cleavage have failed to yield any active polymerase.** [Emphasis added.]

(WO 97/12033, at page 2, line 25 to page 3, line 3.)

The long felt-need is evident based on the medical importance of HCV, the desirability of an assay to screen for a HCV RNA-dependent RNA polymerase inhibitor, and the time difference between prior art speculations concerning HCV RNA-dependent RNA polymerase and applicant's priority application. Speculations concerning the HCV protein responsible for RNA-dependent RNA polymerase are noted at least as early as 1990. (Miller et al., (Proc. Natl. Acad. Sci. USA, March 1990, 87(6), 2057-

2061).) The present application has a priority date of May 25, 1995, which is more than five years after Miller et al. was published.

Accordingly the claims are in condition for allowance. Please charge deposit account 13-2755 for fees due in connection with this amendment. If any time extensions are needed for the timely filing of the present amendment, applicants petition for such extensions and authorize the charging of deposit account 13-2755 for the appropriate fees.

Respectfully submitted,

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